DAKEWE CoE
Flow cytometry Training Core

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STANDARD OPERATING PROCEDURE

Training & Education

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Table of Contents

Section	Page
1. Policies	2
2. Purpose	2
3. Responsibilities	2
4. Procedures	
4.1. Onboarding	2
4.2. Beckman CytoFLEXS Training	3-4
4.3. Cytek Northern Lights 3L Training	4-6
4.4. BD FACS Aria III SORP Training	6
4.5 Aglient Advanteon Training	7-8
5. Date established and subsequent reviews	8

1. Policies

1.1 All users of the cytometers in the DAKEWE CoE Flow cytometry Training Core must receive training in principles and instrumentation, instrument operations, quality control (QC), reagents and panel design, acquisition and measurements, analysis and statistics prior to beginning work in this shared resource laboratory (SRL). Users who wish to conduct experimentsof normal operating hours (Monday through Fridays, 9AM-5PM) must receive advanced training to ensure independence.

2. Purpose

2.1 To ensure competency in both the theoretical and technical aspects of flow cytometry as the first step fo promote rigor and reproducibility in flow cytometry experiments.

2.2 Prevent misuse of equipment.

2.3 To learn best practices in the field of flow cytometry.

3. Responsibilities

3.1 Users may not schedule any of the cytometers without first receiving equipment specific training.

3.2 The DAKEWE CoE Flow cytometry Training Core Training Supervisor set all training requirements which are reviewed and endorsed by Core Director

4. Procedures

4.1 Onboarding

4.1.1 Users contact Core director, Tingting Wang, either by email or in person and specify which equipment on which they wish to receive training.

4.1.2 Core manager emails Onboarding Document to customer. The Onboarding document contains the following information:

4.1.2.1 How to apply for a training course and access scheduling calendars

4.1.2.2 Rates for all instruments

4.1.2.3 Information on capabilities of each instrument in the Core.

4.1.2.4 Users Policies

4.2 Beckman CytoFLEX S Training

4.2.1 Review Hands-On training document : CytoFLEX Startup User Guide,you can visit the webpage:https://www.beckman.com/flow-cytometry/research-flow-cytometers/cytoflex/cytoflex-startup-guide.

4.2.2 Schedule a consultation with Core director to discuss the specifics of your experiment and get recommendations on which instrument to use, sample processing and assay specific best practices.

4.2.3 Schedule an in-person, hands-on, equipment-specific training on the Beckman CytoFLEXS in CoE Flow cytometry Training Core (based on staff availability.) This training takes about one hour and covers the following topics:

4.2.3.1 Setting up an new experiment in CytExpert Software

4.2.3.1.1 Adding and naming experiment and tubes

4.2.3.1.2 Setting up workspace – select the desired channel and type in reagent name in the label coulumn.

4.2.3.1.3 Creating appropriate plots and gates and stopping criteria in the workspace.

4.2.3.2 Adjusting voltages and thresholds

4.2.3.3 Instrument Operation and monitoring

4.2.3.3.1 Loading and removing a tube from the SIT

4.2.3.3.2 Flow rate adjustment optimization

4.2.3.3.3 Monitor fluidics and signal stability with a time plot

4.2.3.4 Data acquisition and recording

4.2.3.5 Running compensation

4.2.3.6 Data export

4.2.3.7 Cleaning the instrument after use

4.2.3.8 After completing training and demonstrating proficiency at the above listed tasks, users may then schedule time on and use the CytoFLEX S. For their first run, the Core director/staff is on-site to assist, answer questions and verify that the user is competent to use the instrument independently moving forward.

4.3 Cytek Northern Lights 3L Training

4.3.1 Review Hands-On training document：Cytek Spectral Cytometer Administrator's Guide,you can visit the webpage:https://cytek-web.s3.amazonaws.com/cytekbio.com/documentation-center/Guides/N9-20113_Administrator's+Guide.pdf

4.3.2 Spectral Best Practices Training – Because of the number of parameters available and the sensitivity of the instrument, it is absolutely crucial to follow best practice in panel design and reference control optimization.

4.3.2.1 Review the following videos:

4.3.2.1.1 Applying Panel Design Principles

4.3.2.1.2 Best Practices for Reference Controls Optimization

4.3.2.1.3 SpectroLearn Panel Design Best Practices

4.3.2.2 Consult with Core director to plan panel design, QC reference controls, QC unmixing and autofluorescence extraction options as needed.

4.3.3 Cytek Northern Lights 3L Hands on Training

4.3.3.1 Schedule an in-person, hands-on, equipment-specific training on the Cytek Northern Lights in CoE training Center (based on staff availability.) This training takes about one hour and covers the following topics:

4.3.3.1.1 Cytek lasers and filter arrays

4.3.3.1.2 Setting up an experiment in SpectroFlow including the following:

4.3.3.1.2.1 checking waste and sheath levels,and then Refilling sheath and emptying waste

4.3.3.1.2.2 Running QC

4.3.3.1.2.3 Create and open an experiment

4.3.3.1.2.4 Choose fluorescent tags

4.3.3.1.2.5 Create groups, tubes plate layout

4.3.3.1.2.6 Labeling fluorophores with marker names

4.3.3.1.2.7 Setting stopping criteria

4.3.3.1.2.8 Loader settings

4.3.3.1.2.9 Add keywords

4.3.3.1.2.10 Worksheet elements on both raw and unmixed worksheets

4.3.3.1.3 Adjusting FSC, SSC and SSC-B voltages and adjusting FSC threshold

4.3.3.1.4 Data acquisition and recording

4.3.3.1.5 Monitor fluidics and signal stability with a time plot

4.3.3.1.6 Unmix data

4.3.3.1.6.1 Running reference controls,

4.3.3.1.6.2 Checking spectral signatures of reference controls

4.3.3.1.6.3 Unmixing

4.3.3.1.6.4 Checking quality of unmixing

4.3.3.1.6.5 Troubleshoot unmixing

4.3.3.1.7 Exporting data and managing data on instrument

4.3.3.1.8 Running cleaning protocol after use

4.3.3.1.9 End of day shut down and power off instrument

4.3.3.1.10 After completing training and demonstrating proficiency at the above listed tasks, users may then schedule time on and use the Cytek. For their first run, the FCCSF director is on-site to assist, answer questions and verify that the user is competent to use the instrument independently moving forward.

4.4 BD FACS Aria III SORP Training Sorting on the Aria is a high skill level, so sorts will be set up and run by facility staff in most cases. Users should have basic flow cytometry knowledge, a basic theory of droplet-based sorting, how to select nozzle size and sample prep for sorting.

4.4.1 Complete online training

4.4.1.1 Flow cytometry training course on CoE training center website if not already completed.

4.4.2 Consult with Core director/staff

4.4.2.1 Review Reference Guide for Cell Sorting Sample Preparation and decide on nozzle size, event rate, sample and collection vessel type, sort temperature, sort buffer and collection media/buffer that are best for your sample type.

4.4.2.2 Users may then schedule time and use the BD FACS Aria III SORP based on facility director/staff availability .

4.5 Aglient Advanteon Training

4.5.1 Review Hands-On training document: NovoCyte Advanteon Flow Cytometer Operator's Guide, you can visit the webpage:

https://www.agilent.com.cn/cs/library/usermanuals/public/150217-NovoCyte%20Advanteon%20Flow%20Cytometer%20Operator%20Guide.pdf . Schedule a consultation with Core director to discuss the specifics of your experiment and get recommendations on which instrument to use, sample processing and assay specific best practices.

4.5.2 Schedule an in-person, hands-on, equipment-specific training on the NovoCyte Advanteon in CoE Flow cytometry Training Core (based on staff availability.) This training takes about one hour and covers the following topics:

4.5.2.1 Setting up an new experiment in NovoExpress Software

4.5.2.1.1 Adding and naming experiment and sample tubes

4.5.2.1.2 Setting up workspace – select mixing interval, duration, speed and acceleration in the Plate Manager window.

4.5.2.1.3 Select your parameters for this experiment in parameters window.

4.5.2.1.4 Creating appropriate plots and gates and stopping criteria in the workspace.

4.5.2.2 Adjusting thresholds, and use the default Gain generally.

4.5.2.3 Instrument Operation and monitoring

4.5.2.3.1 Loading and removing a tube from the SIT

4.5.2.3.2 Flow rate adjustment optimization

4.5.2.3.3 Monitor fluidics and signal stability with a time plot

4.5.2.4 Data acquisition and recording

4.5.2.5 Running compensation

4.5.2.6 Data export

4.5.2.7 Cleaning the instrument after use

4.5.2.8 After completing training and demonstrating proficiency at the above listed tasks, users may then schedule time on and use the NovoExpress. For their first run, the Core director/staff is on-site to assist, answer questions and verify that the user is competent to use the instrument independently moving forward.

5. Date established and subsequent reviews (annually at a minimum)

Revision	Review or Revision Date	Description of Changes	Reviewed By
1.0	01/01/25	New SOP	